10/035.093. 11.24.09

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 September 2001 (27.09.2001)

PCT

(10) International Publication Number
WO 01/71344 A2

(51) International Patent Classification[7]: G01N 33/00

(21) International Application Number: PCT/US01/07022

(22) International Filing Date: 6 March 2001 (06.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/527,801 17 March 2000 (17.03.2000) US

(71) Applicant *(for all designated States except US)*: **QUANTUM DESIGN, INC.** [US/US]; 11578 Sorrento Valley Road, Suite 30, San Diego, CA 92121 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **LA BORDE, Ronald, T.** [US/US]; 8163 Royal Gorge Drive, San Diego, CA 92119 (US).

(74) Agent: **BAKER & MAXHAM**; Lawrence A. Maxham, 750 B Street, Suite 3100, San Diego, CA 92101 (US).

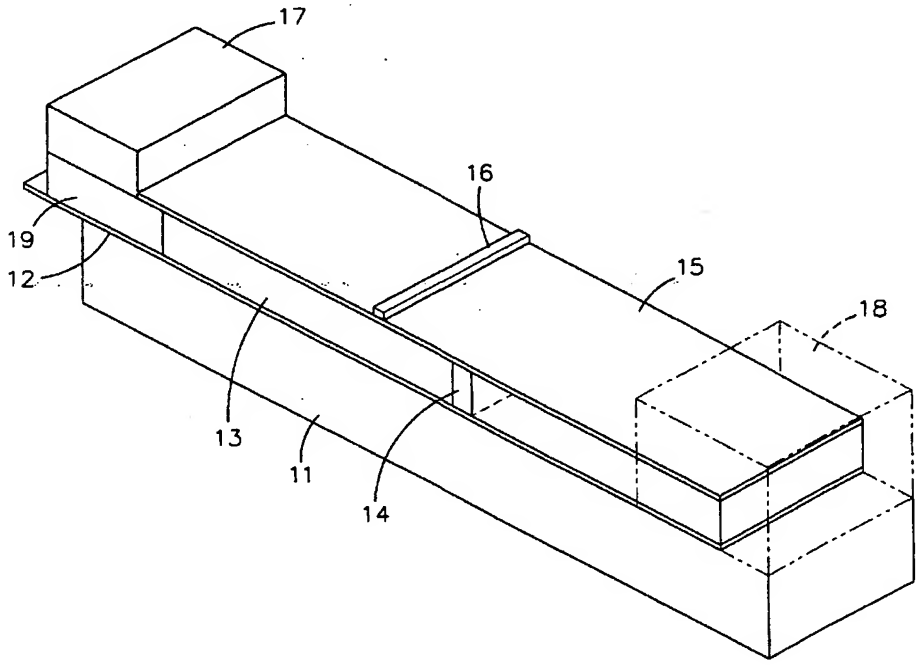
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

[Continued on next page]

(54) Title: IMMUNOCHROMATOGRAPHIC ASSAY METHOD AND APPARATUS



(57) Abstract: An immunochromatographic assay employing superparamagnetic particles to lable the target analytes. An opaque cover (15) prevents misinterpretive readings in field situations and provides a protective surface on the porous membrane (13). Additional features include separability of the test strip from any backing (14) or housing which is configured to support the strip, and that quantitative measurements of the target analytes are easily and accurately made by means of an electromagnetic reader device (21).

WO 01/71344 A2

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

IMMUNOCHROMATOGRAPHIC ASSAY METHOD AND APARATUS

TECHNICAL FIELD

This invention relates generally to immunoassays, and more specifically to an improved chromatographic assay, often referred to as a lateral flow assay, having a test strip employing susperparamagnetic particles as the labels for the analytes to be detected, where, as an additional feature, the analytical strip is removable for reading the quantity of analytes captured therein and for archival purposes.

BACKGROUND ART

Various chromatographic immunoassay techniques have been available for many years. One common aspect of known devices, particularly in the lateral flow technology, is that the assay is read visually, that is, by means of one or more optically readable lines on a test strip, typically held in a carrier, which may have various configurations. One end of the test strip is exposed to the sample, normally a body fluid of some type, being tested for the particular target analytes of interest. It is known that particular analytes are indicative of particular biological, environmental, and biohazard conditions, among others. For example, urine may be tested for pregnancy or ovulation and if the target analytes are present, the test is positive. Body fluids may be tested for the presence of other analytes indicative of biological conditions or they may be indicative of the presence of substances, such as drugs. Another example would be for testing water for contaminates. Examples of lateral flow assay methods and apparatuses, where the reading is normally conducted optically, are shown in U. S.

patents 4,632,901; 5,591,645; 5,602,040; 5,622,871; 5,714,389; 5,798,273; 5,879,951; and 5,958,790.

A different technology is employed in other types of biological technologies employing magnetic particles or micobeads, sometimes more specifically termed superparamagnetic iron oxide impregnated polymer beads. These beads are employed to bind with the target analytes in the sample being tested and are then typically isolated or separated out magnetically. Once isolation has occurred, other testing may be conducted, including observing particular images, whether directly optically or by means of a camera. Examples of these technologies are disclosed in U. S. patents 3,981,776; 5,395,498; 5,476,796; 5,817,526; and 5,922,284. Another apparatus for detecting target molecules in a liquid phase is shown in U. S. patent 5,981,297 where magnetizable particles are employed and the output of magnetic field sensors indicates the presence and concentration of target molecules in the sample being tested. Other examples to sense magnetically using physical forces are disclosed in U.S. patents 5,445,970; 5,981,297 and 5,925,573.

There are several limitations or disadvantages to the known optically detected assays. Because they are optical, only surface changes (coloration, typically) can be detected. The target analytes may be in the sample solution but of such a low concentration that only a relatively few are captured in the capture zone in the porous membrane of the assay. This provides a faint or even non-optically detectable line, and a resultant false negative reading. Quantitative assessments are really only an estimation based on color intensity of the detection line. Because the prior art assays are

optically read, they are subject to contamination by exposure, and light-caused degradation. Optical assays have a limited archival shelf life.

None of the known prior art employs magnetic particles in conjunction with lateral flow assay technology.

5

DISCLOSURE OF INVENTION

Broadly speaking, the invention relates to lateral flow immunoassay technology employing superparamagnetic particles as the labels for the analytes to be detected. The bound complexes of labeled particles and analytes are captured in predetermined areas
10 or regions on the test strip and the presence and quantity of labeled analytes are then readable by magnetic means. An advantageous additional feature of the invention is that the test strip can be removable from the support member for archival purposes or for reading by an appropriate magnetic sensing device, or both.

More specifically, the invention is a lateral flow assay device for quantitative
15 detection of target analytes in a sample, the device comprising: an assay support member having a first end and a second end; a sample receiving element at one end of the support member for introduction of the sample to be analyzed to the device; and an immunoassay test strip comprising: a porous analytical membrane removably mounted adjacent to and generally parallel with the support member, the analytical
20 membrane having a first end and a second end; at least one capture region in the analytical membrane intermediate the first and second ends thereof, the at least one capture region being configured to capture labeled analytes moving from the first end of the analytical membrane toward the second end of the analytical membrane; and a backing member between the analytical membrane and the support member to facilitate

removal of the analytical membrane from the support member for reading the assay and for archiving the test strip.

The invention is also directed to a method for conducting a lateral flow immunoassay quantitative detection of target analytes in a sample, the method comprising: applying the sample to one end of the porous membrane of a lateral flow test strip; coupling superparamagnetic conjugate particles residing in the test strip at the one end, the superparamagnetic particles being treated to bind with any target analyte in the sample; capturing the bound complexes of analyte and susperparamagnetic particles in the capture region of the porous membrane as the sample and bound complexes move through the porous membrane by capillary action; reading the quantity of labeled analytes in the capture region; and providing an output representative of the quantity of labeled analytes in the capture region

A relatively standard lateral flow assay structure is employed but the invention greatly improves the sensitivity of the device over known lateral flow techniques. It provides a very rapid (a few seconds) measurement of the analytical region in the test strip. There are many advantages of using magnetic particles over known colored particles or other optical indicators in the prior art. These include linearity because magnetic detection is linear with respect to the amount of magnetic material present over a wide range, through at least four orders of magnitude. Time stability is also significant because magnetic particles are stable. The developed assay is available to be archived and retested as necessary. Further, magnetic particles are generally inert to biological systems and the environment so they not only remain stable, they are environmentally and biologically safe. Further, magnetic particles are already in

widespread use throughout the diagnostics industry with other technologies so they are readily available. Other benefits of magnetic detection are that since the particles are superparamagnetic, they are magnetic only when in a magnetic field. This allows them to be freely manipulated in solution without aggregating.

Another significant advantage over the prior art optical lateral flow devices is that with this invention the total amount of analytes in the capture region of the test strip is measured as a single mass in one volumetric measurement by magnetic means. The permeability of magnetic fields is such that any analyte contained within the active region of the detector will be measured. This contrasts with optical sensing techniques in which only reporter-analyte interactions on or very near the surface are detectable. In this invention the strength of the magnetic signal increases directly with the mass of iron involved. This inherent linearity of magnetic detection contributes to sensitivity, accuracy and dynamic range. Finally, superparamagnetic particles are physically similar to collodial gold with regard to size, and may be easily adapted to a wide range of lateral flow assays. It is noted that collodial gold, as well as fluorescent latex particles, are typically employed in the prior art optically sensed immunological assay techniques.

In lateral flow technology, at one end of the porous membrane (the active part of the test strip) is the sample introduction area conventionally comprising a sample pad and a conjugate pad. In the prior art, the conjugate pad was the source of freely moveable colored particles, typically gold sols from collodial gold, or fluorescent latex particles. In the present invention, the moveable particles are the superparamagnetic particles which label the target analytes from the sample being introduced through the

sample pad. The sample, together with the bound magnetic particle labels and target analytes, move with capillary action along the porous membrane and are captured in a predefined location called a capture region or capture zone. There may be more than one capture zone to enable multiplexing, that is, testing for more than one type of analyte at the same time in the same test strip. Excess analytes and the carrying liquid continue to move on through the capture zone to the other end of the porous membrane, sometimes forming a control line or zone separate from the capture zone. An added feature is that typically a wicking pad is mounted on the far end of the porous membrane to enhance the capillary action which drives the flow from the introduction at one end of the porous membrane through the entire length of the membrane.

The porous membrane typically is mounted on a relatively rigid support or base member, but in an advantageous embodiment a separation sheet, or adhesive layer, exists between the base member and the porous membrane. This enables very easy removal of the test strip, which normally would include the separation sheet, so that the test strip is a very thin element which may be magnetically sensed in an appropriate device. Since optical means are not employed and color at the capture zone has no meaning, the top of the porous member is preferably covered by another protective sheet or membrane which is not transparent. It may be completely opaque. This top sheet may also include pre-printed standards, which are employed for calibrating purposes so that the magnetic detector can be calibrated for each test to ensure complete accuracy. The protective sheet may not be a separate element in some cases, but may only be the upper surface of the membrane properly treated to function as a protective sheet or surface.

BRIEF DESCRIPTION OF DRAWING

The objects, advantages and features of this invention will be more readily appreciated from the following detailed description, when read in conjunction with the accompanying drawing, in which:

Fig. 1 is a schematic perspective, partially phantom, view of an embodiment of the invention;

Fig. 2 is a schematic side view of the Fig. 1 embodiment;

Fig. 3 is a schematic side view of a preferred embodiment of the invention;

Fig. 4 is a top view of the Fig. 3 embodiment;

Fig. 5 shows how the test strip of Fig. 3 is removed;

Fig. 6 is a perspective of a reader device used with the assay apparatus of Fig. 1 or 3; and

Fig. 7 schematically shows how the assay strip is read by a magnetic reader device.

BEST MODE FOR CARRYING OUT THE INVENTION

With reference now to the drawing, and more particularly to Figs. 1 and 2, there is shown an embodiment of the present invention, a lateral flow test strip for an immunochromatographic assay. A relatively rigid assay support member 11 serves as a base and provides support for the test strip, which in this embodiment is removable from the support. On top of the support member is backing member 12 on which is mounted porous membrane 13. The backing member is removably adhered to the support, or it may itself be an adhesive layer. Within the porous membrane is capture zone 14. The capture zone is formed by striping with antigens or antibodies, for example, as is well known in the

art. On top of the porous membrane is non-light transmissive cover or surface 15. The cover or surface may be considered to be optically opaque, at least to the extent that capture zone indicia would not be visible through cover 15. The protective membrane may be made of plastic, glass or paper, for example, or any practical combination thereof. A printed standard or calibration line 16 is situated on top of the cover and provides information utilized by the assay reader after the test has been accomplished. This is contemplated to be a magnetic stripe with the information the reader needs.

At the left end, as shown in Figs. 1 and 2, is sample pad 17, through which an analyte-containing sample solution 20 is administered to the porous membrane, with analytes 20a shown in the sample pad. The sample pad may also include conjugate pad 19 which is in communication with the porous membrane. Within the conjugate pad are superparamagnetic beads or particles which are coupled with antibodies, the combination of a bead and an antibody being referred to as a conjugate, a plurality of them being labeled with reference numeral 20b. These conjugates are configured to combine with target analytes in the sample solution in a known manner to create a sandwich assay, well known in the art, where the beads provide labels for the target analytes. Competitive assay techniques, also well known in the art, could also be employed.

The porous membrane will, as a feature of some embodiments, have a wicking pad 18 at the opposite, or right, end of the test strip. This is a conventional element.

The operation of the lateral flow assay is well known. The labeled analytes (in a sandwich assay, for example) move by capillary action from the left to the right as seen in Fig. 1. Labeled analytes are captured in the capture zone where reading of the assay results is accomplished. Wicking pad 18 enhances capillary flow in the porous membrane by

"pulling," or "driving" the fluid through the porous membrane. A typical material from which the porous membrane is made is nitrocellulose.

While the capillary action and the existence of a capture zone are well known and conventional, the manner in which the described embodiments of the invention detect the presence and the quantity of the target analytes differs greatly from the prior art. The known lateral flow assays depend upon color or fluorescence to provide a visual or optical indication of the presence of target analytes in the capture zone, but the ability of optical techniques to detect the presence of the target analytes is limited. A relatively low concentration of target analytes in the sample can result in so few captured analytes as to be optically undetectable on the surface of the porous membrane at the capture zone. Further, the optical intensity of the capture zone with the captured analytes is only a rough function of the quantity of target analytes captured. However, there is no way to accurately measure the total quantity of captured analytes within the capture zone because only the surface is optically readable.

The present embodiment provides greatly enhanced sensitivity and quantitative accuracy because the magnetic labeled analytes in the capture zone are detectable by a suitable magnetic detector to the extent of the target analytes within the entire volume of the capture zone.

Additional features may be added, including additional capture zones 14 (two are shown in Figs. 3 and 5) and additional calibration lines 16. In Figs. 3-5 there are four calibration lines, one before and one after each capture zone. There could be several capture zones and equivalent calibration lines.

A significant aspect of an embodiment of the invention is the means and manner of magnetically reading the assay. A magnetic reader 21 of the type contemplated is shown in Fig. 6. This employs the technology disclosed in PCT publication WO 99/27369, to determine the presence of target analytes and their quantity. The reader of the present invention is contemplated to be portable, that is, approximately pocket size, so that it is easily employed in the field. It will provide accurate assay readings even under stressful conditions and in poor light. The apparatus of Fig. 6 has a narrow slit opening 22 into which an assay strip can be inserted for reading by electromagnetic means. The analyte quantity may be shown in window 23, which could be an LED or an LCD screen, for example. The manner in which the capture zone with analytes bonded therein is read is shown schematically in Fig 7. Lower electromagnetic head 31 has a top surface 32 on which is mounted detection coil 34 and across which strip 13/15 is positioned with capture zone 14 centered over the detection coil. The strip is shown in this example with surface or cover 15 of the strip actually touching the surface of the detection coil. Cover 15 thus protects the delicate structure of the porous membrane during the reading process, as well as long term if the strip is archived. Top magnet 33 is closely adjacent to the opposite side of the strip, which has stripping layer 12 thereon. The structure of the electromagnetic head shown in Fig. 7 is generally equivalent to that in reader 21.

To enable the reader of Fig. 6 to be employed to read the analytes in the capture zone, the test strip is made readily removable from support member 11, and from sample pad 17 and wicking pad 18. As seen in Figs. 3-5, pull tab 24 is secured to cover 15, which is, in turn, secured to porous membrane 13. The porous membrane is removably secured to the support by means of a removable backing 12 which is secured to the bottom of the

porous membrane. It will be noted that the assay is essentially inverted as shown here. The sample could be applied to sample pad 17 with the strip turned over from the position of Figs. 3 and 5. In this reverse position, conjugate pad 19 and wicking pad 18 are positioned between support member 11 and the peelable strip 12, 13, 15. Element 11a may be a non-active polymer fill membrane. Sheet or adhesive 12 is readily removable from the surfaces of elements 18, 19 and 11a.

It is contemplated that the test strip, primarily consisting of the porous membrane and protective surfaces thereof, may be made sufficiently rigid to not need a support member. Such a configuration would not need to be stripped from anything. The reader apparatus might have to be modified somewhat to handle the different configuration of the test strip, but the principle of magnetic reading shown in Fig. 7 still applies.

Fig. 5 shows how the test strip, comprised of the cover, porous membrane and removable backing, is removed from the support member. In actuality, this removed test strip is typically about 3-12 mm wide, and only about 150-500 μm thick. This strip is easily fed into reader 21 for a digital readout, which readout may be shown on the screen or printed on paper in any desired form by reader 21. The exposed test strip is stable and can be archived either before or after being read. Since the superparamagnetic beads are magnetized only during the reading process, the exposed test strip is not subject to degradation. The analytes contained in the capture zone remain there, labeled with the conjugate combination.

Alternative features of the embodiment discussed above contemplate the test strip being slid off the support member or peeled off, either manner of removal being physically possible.

The opaque surface or cover 15 has several positive functions. Contrary to prior art optical lateral flow assays, where very faint lines can easily be misinterpreted in the field, especially in stressful situations or low light conditions, there is no possibility of misinterpretation of test results with this invention. Optically read assays, especially those visually read, are also subject to operator bias. In the present invention the reader reads the total number of labeled analytes in the capture zone without inherent sources of error as mentioned above. Further, the thickness of the opaque cover precisely positions the porous membrane and thus, the capture zone, with respect to the magnet head and detection coil 34. Since the test strip actually touches the detection coil, without the protective surface the porous nitrocellulose membrane would be damaged by rubbing across detection coil 34, thereby possibly producing incorrect or unreliable readings, or both. Although being very thin, in the range of 30-50 μm the cover protects against physical damage and environmental contamination as well as providing precise positioning for accurate electromagnetic readings.

While the present invention has been illustrated and described by means of a specific embodiment, it is to be understood that numerous changes and modifications can be made therein without departing from the scope of the claims and equivalents thereto.

CLAIMS

1. A lateral flow assay device for quantitative detection of target analytes in a sample, said device comprising:

an assay support member (11) having a first end and a second end;

a sample receiving element at (17) one end of said support member for introduction of the sample to be analyzed into said device; and

an immunoassay test strip comprising:

a porous analytical membrane (13) removably mounted adjacent to and generally parallel with said support member, said analytical membrane having a first end and a second end;

at least one capture region (14) in said analytical membrane intermediate said first and second ends thereof, said at least one capture region being configured to capture labeled analytes moving from said first end of said analytical membrane toward said second end of said analytical membrane; and

a backing member between (12) said analytical membrane and said support member to facilitate removal of said analytical membrane from said support member for reading the assay and for archiving said test strip.

2. The device recited in claim 1, and further comprising a protective membrane (15) covering said analytical membrane on the side opposite to said support member, said protective membrane being optically non-transparent.

3. The device recited in claim 2, wherein said protective membrane is formed integrally with said porous membrane.

4. The device recited in claim 2, wherein said protective membrane is formed pursuant to a surface treatment of said porous membrane.

5. The device recited in claim 1, and further comprising a control region in said porous membrane for collection of magnetic conjugates that have passed the capture region to show that said test strip has been used.

6. The device recited in claim 2, and further comprising at least one magnetic calibration line (16) printed on said protective membrane.

7. The device recited in claim 2, wherein said protective membrane is formed of material selected from the group consisting of plastic, glass and paper.

8. The device recited in claim 1, and further comprising superparamagnetic conjugate particles in said sample receiving element, said particles being configured to bind with target analytes in the sample.

9. A lateral flow assay device for quantitative detection of target analytes in a sample, said device comprising:

an assay support member (11) having a first end and a second end;

a sample receiving element (17) near one end of said support member for introduction of the sample to be analyzed to said device; and

an immunoassay test strip comprising:

a porous analytical membrane (13) removably mounted adjacent to and generally parallel with said support member, said analytical membrane having a first end and a second end;

superparamagnetic conjugate particles in said sample receiving element configured to bind with the target analytes in the sample;

a capture region (14) in said analytical membrane intermediate to said first and second ends thereof, said capture region being configured to capture labeled analytes moving from said first end of said analytical membrane toward said second end of said analytical membrane; and

a backing member (12) between said analytical membrane and said support member to facilitate removal of said analytical membrane from said support member for reading the assay and for archiving said test strip.

10. The device recited in claim 9, and further comprising a protective membrane (15) covering said analytical membrane on the side opposite to said support member, said protective membrane being optically non-transparent.

11. The device recited in claim 10, wherein said protective membrane is formed integrally with said porous membrane.

12. An analytical immunoassay apparatus for quantitative detection of target analytes in a sample, said apparatus comprising:

an assay support member (11) having a first end and a second end;

a sample receiving element (17) near one end of said support member for introduction of the sample to be analyzed to said apparatus;

an immunoassay test strip comprising:

a porous analytical membrane (13) removably mounted adjacent to and generally parallel with said support member, said analytical membrane having a first end and a second end;

superparamagnetic conjugate particles in said sample receiving element configured to bind with the target analytes in the sample;

a capture region (14) in said analytical membrane intermediate to said first and second ends of said analytical membrane, said capture region being configured to capture labeled analytes moving from said first end of said analytical membrane toward said second end of said analytical membrane; and

a backing member (12) between said analytical membrane and said support member to facilitate removal of said analytical membrane from said support member for selectively reading the assay and archiving said test strip; and

a magnetic reader device (21) for determining the presence and quantity of magnetic conjugate particle labeled target analytes in said capture region, said reader device being shaped and configured to receive said test strip after the lateral flow process has been completed.

13. The apparatus recited in claim 12, and further comprising a protective membrane (15) covering said analytical membrane on the side opposite to said support member, said protective membrane being optically non-transparent.

14. The apparatus recited in claim 13, wherein said protective membrane is formed integrally with said porous membrane.

15. A method for conducting a lateral flow immunoassay quantitative detection of target analytes in a sample, a method comprising:

applying the sample (20) to one end of the porous membrane (13) of a lateral flow test strip;

coupling superparamagnetic conjugate particles residing in the test strip at said one end, the superparamagnetic particles being treated to bind with any target analyte in the sample;

capturing the bound complexes of analyte and susperparamagnetic particles in the capture region (14) of the porous membrane as the sample and bound complexes move through the porous membrane by capillary action;

reading the quantity of labeled analytes in the capture region; and

providing an output representative of the quantity of labeled analytes in the capture region

16. The method recited in claim 15, and further comprising removing the test strip from the lateral flow assay device with the bound complexes remaining available to be

selectively stored and sensed as to the quantity of the bound complexes in the capture region.

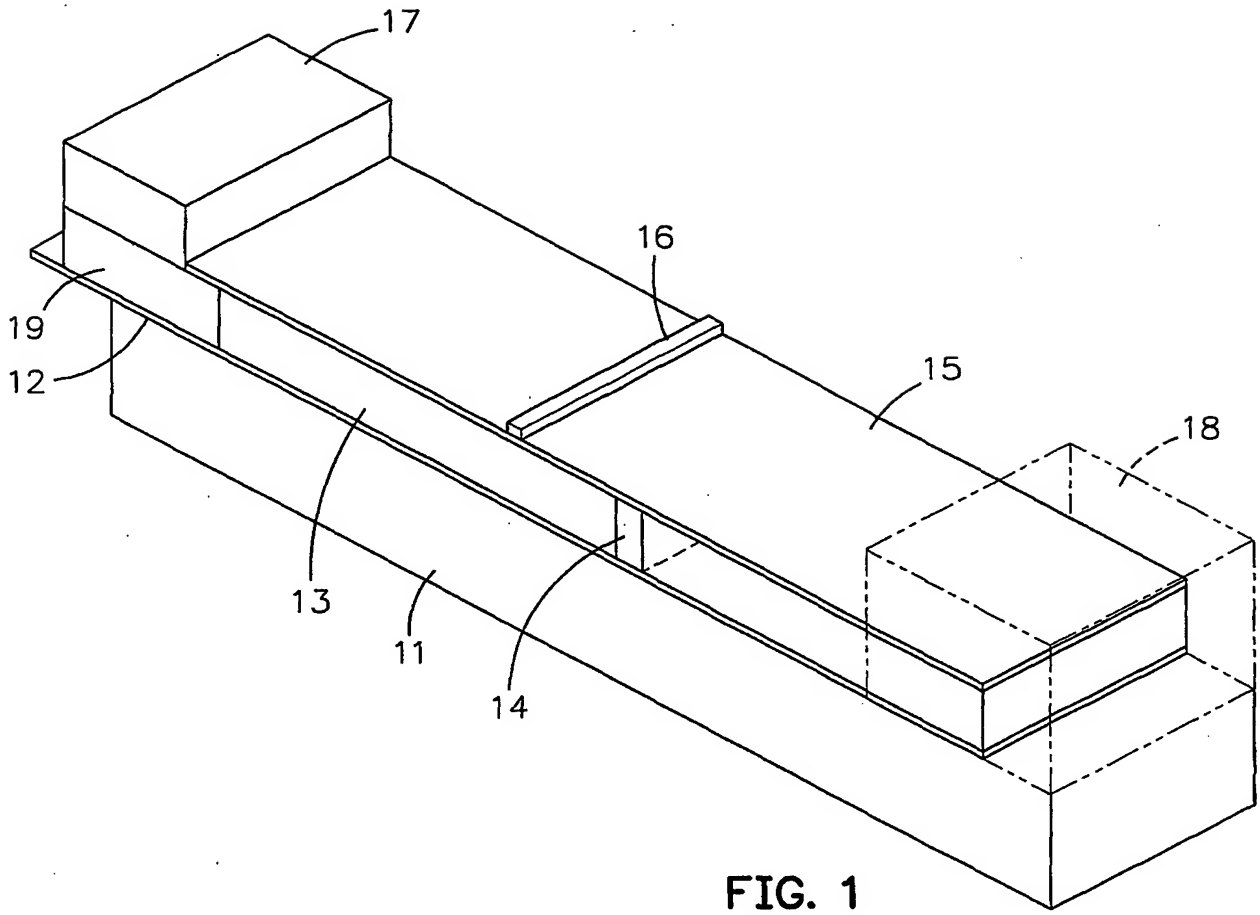


FIG. 1

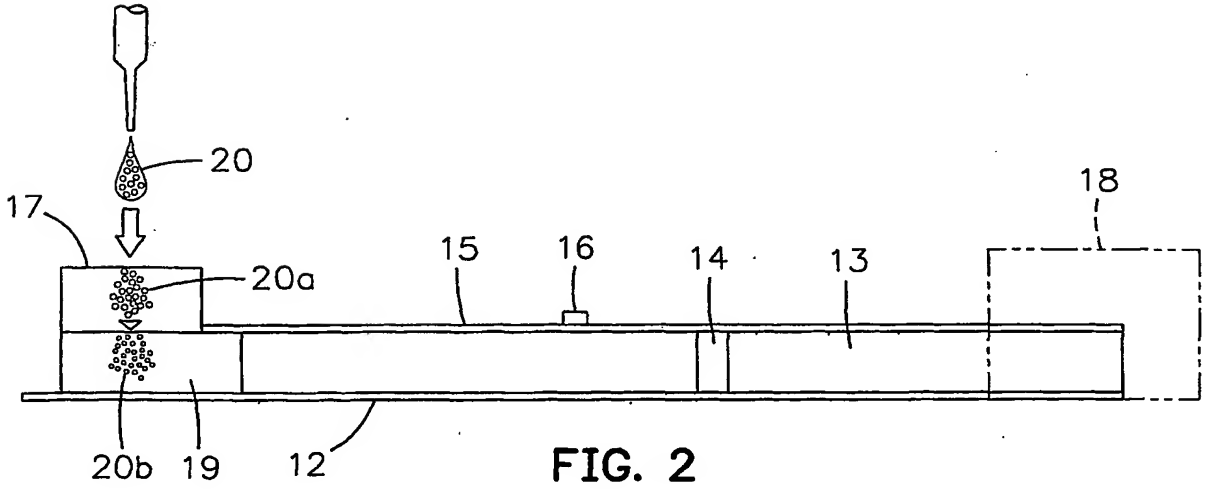


FIG. 2

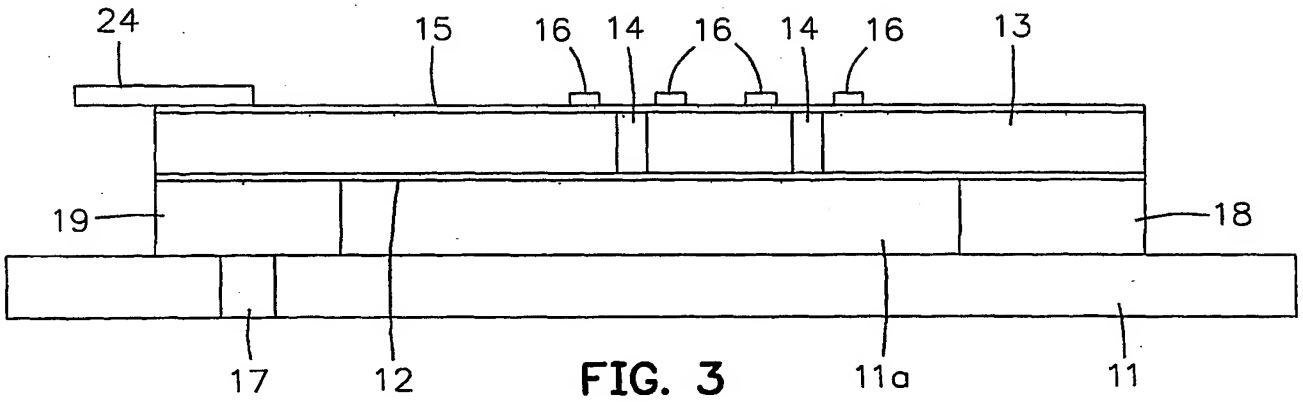


FIG. 3

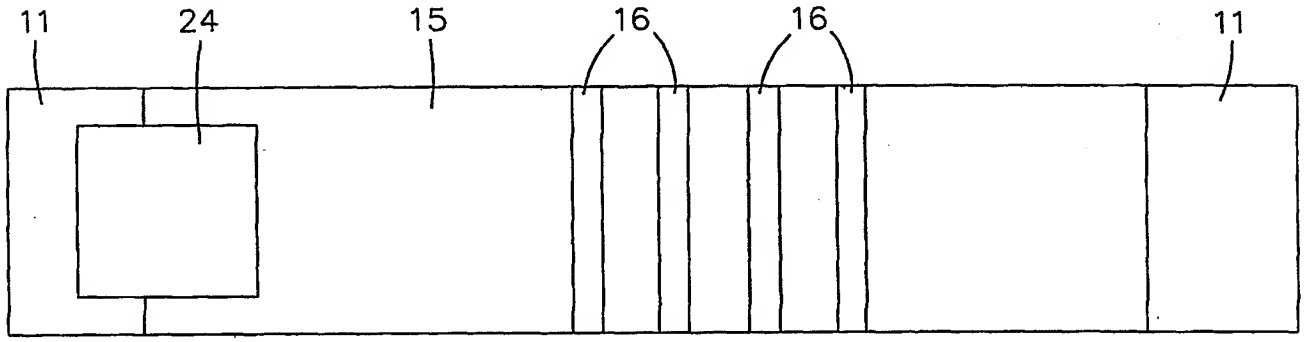


FIG. 4

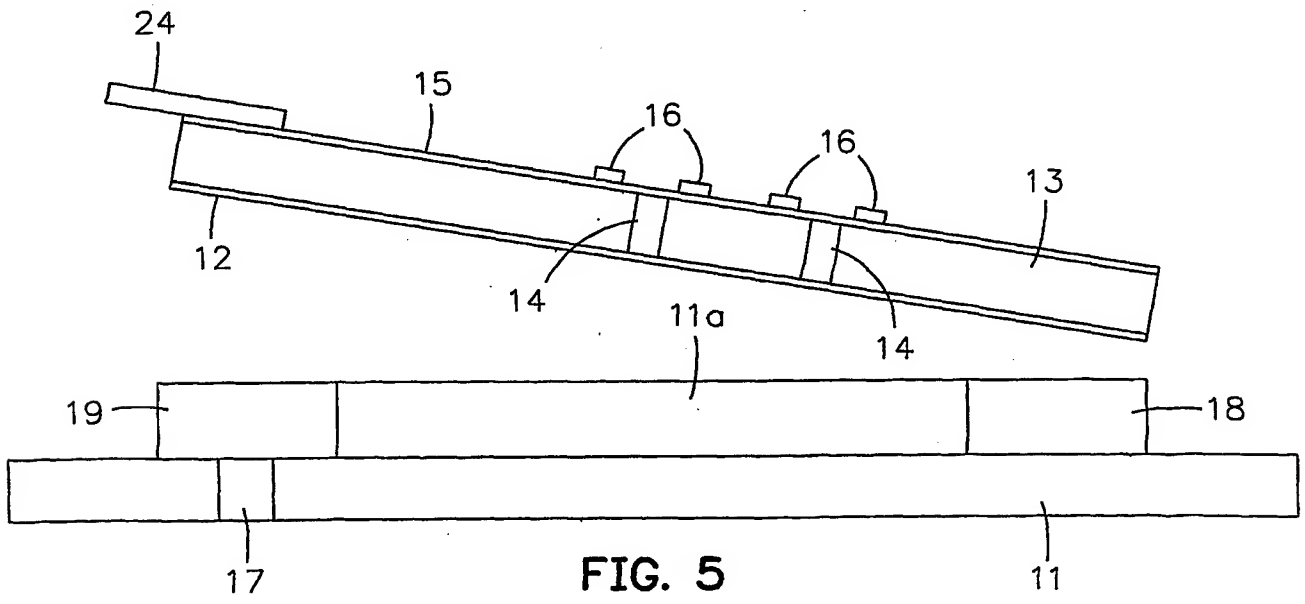


FIG. 5

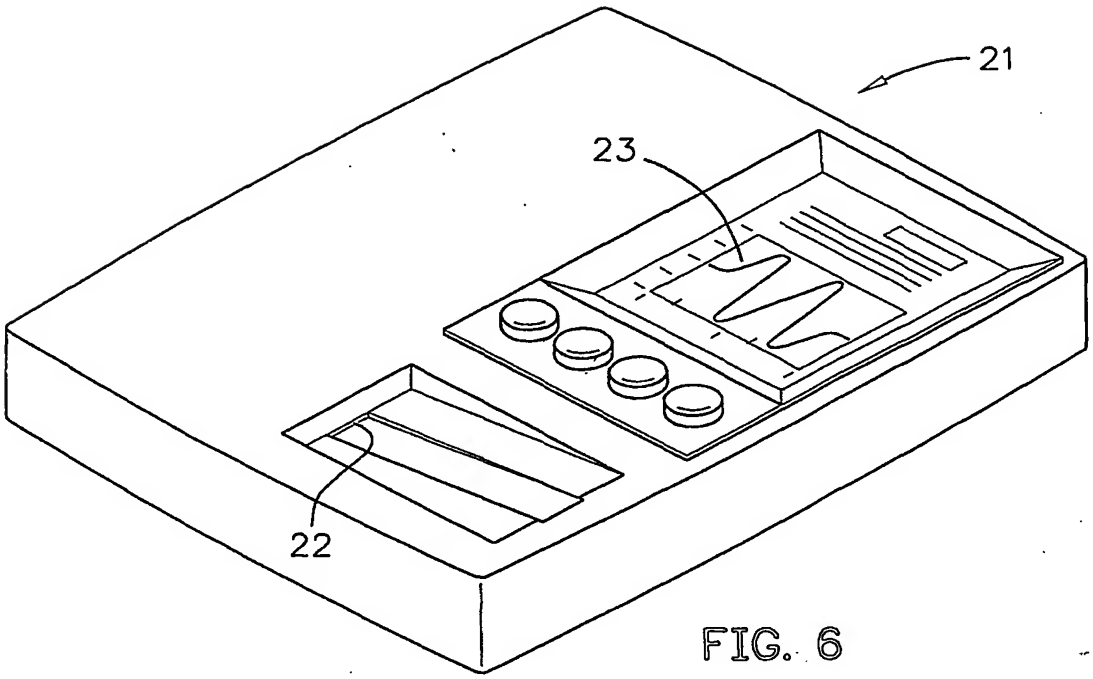


FIG. 6

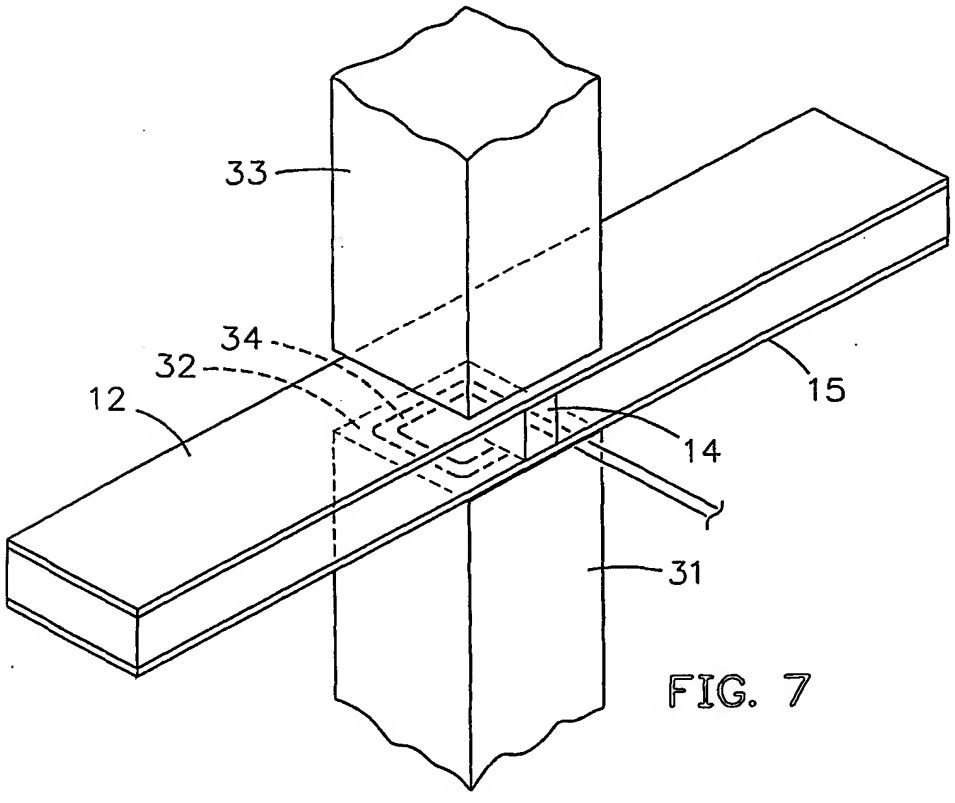


FIG. 7

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 September 2001 (27.09.2001)

PCT

(10) International Publication Number
WO 01/71344 A3

(51) International Patent Classification[7]: G01N 33/558, 33/58

(21) International Application Number: PCT/US01/07022

(22) International Filing Date: 6 March 2001 (06.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/527,801 17 March 2000 (17.03.2000) US

(71) Applicant *(for all designated States except US)*: QUANTUM DESIGN, INC. [US/US]; 11578 Sorrento Valley Road, Suite 30, San Diego, CA 92121 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: LA BORDE, Ronald, T. [US/US]; 8163 Royal Gorge Drive, San Diego, CA 92119 (US).

(74) Agent: BAKER & MAXHAM; Lawrence A. Maxham, 750 B Street, Suite 3100, San Diego, CA 92101 (US).

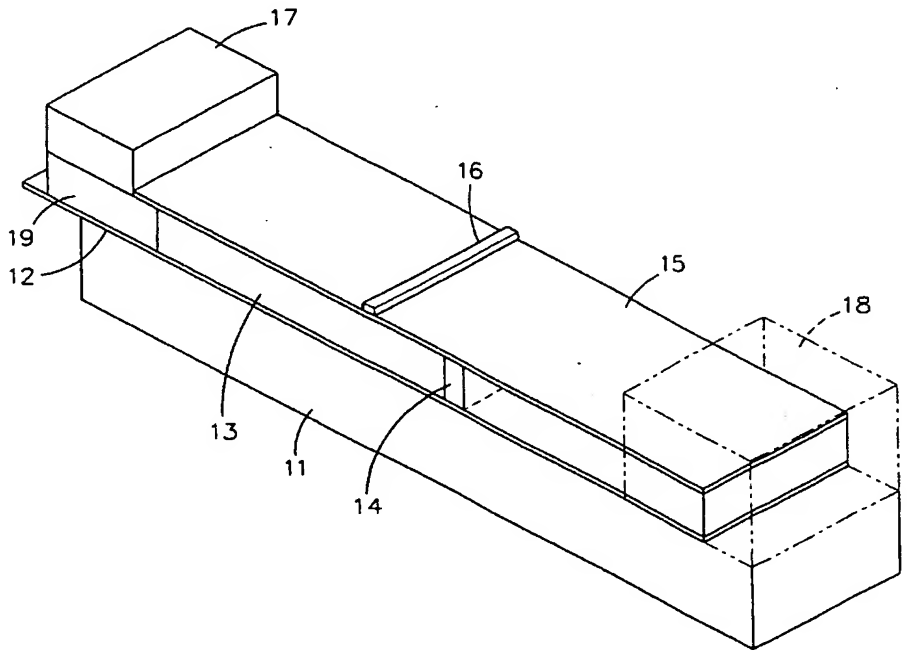
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: IMMUNOCHROMATOGRAPHIC ASSAY METHOD AND APPARATUS



(57) Abstract: An immunochromatographic assay employing superparamagnetic particles to lable the target analytes. An opaque cover (15) prevents misinterpretive readings in field situations and provides a protective surface on the porous membrane (13). Additional features include separability of the test strip from any backing (14) or housing which is configured to support the strip, and that quantitative measurements of the target analytes are easily and accurately made by means of an electromagnetic reader device (21).

WO 01/71344 A3

(88) Date of publication of the international search report:
10 January 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Inter- tional Application No
PC1/US 01/07022

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/558 G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 34150 A (ABBOTT LABORATORIES) 18 September 1997 (1997-09-18) page 7, line 8 - line 13; claims; figure 2	1-16
Y	WO 97 35205 A (SEREX, INC.) 25 September 1997 (1997-09-25) the whole document	1-16
Y	US 5 869 345 A (H. M. CHANDLER) 9 February 1999 (1999-02-09) the whole document	1-16
Y	US 5 968 839 A (J. M. BLATT ET AL.) 19 October 1999 (1999-10-19) the whole document	1-16

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 2001	23/10/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Griffith, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 01/07022

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9734150	A	18-09-1997	WO	9734150 A1	18-09-1997
WO 9735205	A	25-09-1997	AU	2553197 A	10-10-1997
			CA	2249303 A1	25-09-1997
			EP	0888552 A1	07-01-1999
			WO	9735205 A1	25-09-1997
			US	6121008 A	19-09-2000
US 5869345	A	09-02-1999	US	5877028 A	02-03-1999
			US	6168956 B1	02-01-2001
			AU	720394 B2	01-06-2000
			AU	5930796 A	18-12-1996
			CA	2221125 A1	05-12-1996
			CN	1201527 A	09-12-1998
			EP	0855031 A1	29-07-1998
			JP	2001504573 T	03-04-2001
			WO	9638727 A1	05-12-1996
			US	5846838 A	08-12-1998
			AT	177206 T	15-03-1999
			AU	678461 B2	29-05-1997
			AU	6497094 A	24-10-1994
			BG	100104 A	31-05-1996
			BR	9406755 A	02-04-1996
			CA	2158570 A1	13-10-1994
			CN	1124524 A	12-06-1996
			DE	69416828 D1	08-04-1999
			DE	69416828 T2	08-07-1999
			DK	692097 T3	04-10-1999
			EP	0692097 A1	17-01-1996
			ES	2131191 T3	16-07-1999
			FI	954591 A	27-11-1995
			HU	73379 A2	29-07-1996
			JP	8508569 T	10-09-1996
			NO	953872 A	06-11-1995
			NZ	263754 A	24-03-1997
			PL	310953 A1	08-01-1996
			RU	2124729 C1	10-01-1999
			SK	122795 A3	05-06-1996
			WO	9423300 A1	13-10-1994
			US	5468648 A	21-11-1995
			US	5607863 A	04-03-1997
			US	5648274 A	15-07-1997
			US	5998220 A	07-12-1999
			US	6017767 A	25-01-2000
			AT	174432 T	15-12-1998
			AU	665956 B2	25-01-1996
			AU	2185292 A	08-01-1993
			CA	2103052 A1	30-11-1992
			DE	69227834 D1	21-01-1999
			DE	69227834 T2	29-04-1999
			DK	586595 T3	16-08-1999
			EP	0586595 A1	16-03-1994
			EP	0874241 A1	28-10-1998
			ES	2127754 T3	01-05-1999
			FI	9352 A	
US 5968839	A	19-10-1999	CA	2254075 A1	19-11-1998